IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re Application of Group Art Unit: 1623

Keiichirou KAI, et al. Examiner: Keiichirou KAI

Serial No. 10/578,912

Filed: May 9, 2006

For: PROCESS FOR PRODUCING PENTOSE-5-PHOSPHATE ESTER

Honorable Commissioner of Patents and Trademarks
United States Patent and Trademark Office
Washington, D. C. 20231

Sir:

DECLARATION UNDER 37 CFR 1.132

I, Keiichirou KAI, declare and state that:

1. In March 1998, I was graduated from Kyusyu University, Agricultural Chemistry, Agricultural Division. In March 2000, I finished Master Course in Applied Microbiology, Faculty of Molecular Biotechnology in the same University.

Since April 2000, I have been an employee of MITSUI Petrochemical Industries Ltd., which has been named MITSUI Chemicals, Inc. Until March 2003, I had been assigned to Life Science Laboratory and engaged in the research work concerning Biocatalysis. Since April 2003, I have been assigned to group for Biocatalysis and Bioprocess in the Catalysis Science

Laboratory and engaged in research work concerning Molecular Biotechnology.

I am a co-inventor of the invention described in the above-identified application, and have a full understanding of the present invention.

2. I carried out the following experiment in order to demonstrate that acid phosphatase has a substrate specificity.

Experiments and Results

I demonstrate an additional experiment data, Examples A and B. That is:

The target compounds generated by the following Examples A and B were analyzed by the high performance liquid chromatography (hereinafter referred to as HPLC). Conditions of the HPLC analysis are shown below.

Column: Shodex Asahipak NH2P-50 4E (Showa Denko K.K.)

Mobile phase: 50 mM Sodium dihydrogen phosphate

Detector: Differential refractometer

The target compounds were bought, the compounds were analyzed by HPLC, and the working curves respectively were made.

The concentration of phosphoric acid ester was calculated from the analysis result of HPLC in using the working curve.

《For the pentose》

About (1)2-deoxy-D-ribose, (3)D-ribose and (5)D-arabinose, the concentration of each phosphoric acid ester was calculated from the working curve of its the signpost goods respectively.

About (2)2-deoxy-L-ribose, the concentration of phosphoric acid ester was calculated from the working curve of (1)2-deoxy-D-ribose.

About (6)L-arabinose, the concentration of phosphoric acid ester was calculated from the working curve of (5)D-arabinose.

About (4)D-xylose and (7)D-lyxose, there was no standard good, and the peak was not seen. Therefore, each yield was 0.

《For the hexose》

About (1)D-glucose, (2)2-deoxy-D-glucose, (3)D-mannose, (5)D-sorbitol, and (7)D-glucosamine, the concentration of each phosphoric acid ester was calculated from the working curve of its the signpost goods.

About (4)D-galactose, (6)D-myo-inositol, and (8)D-glucono-1,5-lactone, there was no standard good, and the peak was not seen. Therefore, each yield was 0.

Example A

To a solution containing 100mM of acetate buffer (pH=3.5), 700mM of a mixed solution (pH3.5) of a pyrophosphoric acid and a potassium pyrophosphate, and 100mM of various pentoses was added a bacterial cell solution to 0.73U/mL (0.5mg wer bacterial cell / mL) using the culture bacterial cell prepared in Reference Example 1. The resulting mixture was reacted at 30℃. The reaction solution was analyzed by HPLC.

The results thereform were shown in Table.A.

Table.A

(1) 2-deoxy-D-ribose

Reaction time	2-deoxy-D-ribose-5-phosphate
1hr	0.73mM
3hr	2.94mM
6hr	6.45mM
12hr	10.43mM
24hr	9.64mM

(2) 2-deoxy-L-ribose

Reaction time	2-deoxy-L-ribose-5-phosphate
1hr	1.13mM
3hr	2.97mM
6hr	7.07mM
12hr	9.69mM
24hr	9.39mM

(3) D-ribose

Reaction time	D-ribose-5-phosphate
1hr	1.62mM
3hr	4.66mM
6hr	7.51mM
12hr	13.45mM
24hr	9.78mM

(4) D-xylose

Reaction time	D-xylose-5-phosphate
1hr	0mM
3hr	0mM
6hr	0mM
12hr	0mM
24hr	0mM

(5) D-arabinose

Reaction time	D-arabinose-5-phosphate
1hr	0.43mM
3hr	1.06mM
6hr	1.88mM
12hr	3.34mM
24hr	2.51mM

(6) L-arabinose

Reaction time	L-arabinose-5-phosphate
1hr	0.22mM
3hr	0.59mM

6hr	1.15mM
12hr	2.22mM
24hr	1.60mM

(7) D-lyxose

Reaction time	D-lyxose-5-phosphate
1hr	0mM
3hr	0mM
6hr	0mM
12hr	0mM
24hr	0mM

From the results of the above Examples A, 2-deoxyribose, D-ribose and arabinose can be phsphorylated, on the other hand D-xylose and D-lyxose can not be phospharylated from various pentoses.

Example B

To a solution containing 100mM of acetate buffer (pH=3.5), 200mM of a mixed solution (pH3.5) of a pyrophosphoric acid and a potassium pyrophosphate, and 100mM of various hexoses was added a bacterial cell solution to 0.73U/mL (0.5mg wer bacterial cell / mL) using the culture bacterial cell prepared in Reference Example 1. The resulting mixture was reacted at 30℃. The reaction solution was analyzed by HPLC. The results thereform were shown in Table.B.

Table.B

(1) D-glucose

Reaction time	D-glucose-6-phosphate
1hr	34.3mM
2hr	51.6mM
4hr	67.2mM
8hr	71.3mM
12hr	64.5mM

(2) 2-deoxy-D-glucose

Reaction time	2-deoxy-D-glucose-6-phosphate
1hr	40.0mM
2hr	53.8mM
4hr	56.1mM
8hr	21.5mM
12hr	1.5mM

(3) D-mannose

Reaction time	D-mannose-6-phosphate
1hr	9.0mM
2hr	14.8mM
4hr	26.2mM
8hr	10.7mM
12hr	1.1mM

(4) D-galactose

Reaction time	D-galactose-6-phosphate
1hr	0mM

2hr	0mM
4hr	0mM
8hr	0mM
12hr	0mM

(5) D-sorbitol

Reaction time	D-sorbitol-6-phosphate
1hr	8.3mM
2hr	14.6mM
4hr	23.5mM
8hr	13.9mM
12hr	7.1mM

(6) D-myo-inositol

Reaction time	D-myo-inositol-phosphate
1hr	0mM
2hr	0mM
4hr	0mM
6hr	0mM

(7) D-glucosamine

Reaction time	D-glucosamine-6-phosphate
1hr	33.1mM
2hr	48.2mM
4hr	63.7mM
8hr	69.1mM
12hr	63.5mM

(8) D-glucono-1,5-lactone

Reaction time	D-glucono-1,5-lactone-6-phosphate
1hr	0mM
2hr	0mM
4hr	0mM
8hr	0mM
12hr	0mM

From the results of the above Examples B, D-glucose, D-mannose, D-sorbitol and D-glucosamine can be phsphorylated, on the other hand D-galactose, D-myo-inositol and D-glucono-1,5-lactone cannot be phsphorylated from various hexoses.

The undersigned declares further that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

This day of November 12, 2008

Keiichirou KAI